Report for 2003NJ42B: Microbial respiration of arsenic and selenium

- Conference Proceedings:
  - Narasingarao, P and Häggblom , MM. (2004). Physiological Characterization of a Dissimilatory Selenate Reducing Bacterium Strain AK4OH1. in American Society for Microbiology General Meeting 2004.

Report Follows

Project Information

Problem and Research Objectives

Arsenic and selenium though naturally present in the earth's crust become very toxic when their oxyions gain entry into water systems. Oxidation-reduction reactions play a major role in increasing the mobility of these elements whereby they enter water systems. Previous studies indicate that microorganisms mediate these redox reactions using them as alternate electron acceptors during respiration by the process of dissimilatory arsenate or selenate reduction. Both these elements have gained importance in recent years in terms of their toxicity because of human impact on the lithosphere, which has resulted in large-scale release of toxic forms of arsenic and selenium.

Arsenic is widely distributed throughout the earth's crust and is introduced through the dissolution of minerals and ores and ground water levels get elevated due to erosion from local rocks. It is also used in alloying agents, wood preservatives, and pesticides and is also released during the combustion of fossil fuels followed by atmospheric deposition. Seleniferous soils and fossil fuels constitute natural selenium sources; anthropogenic sources such as the combustion of fossil fuels, runoff from irrigated seleniferous soils and draining from mines also add selenium into the environment.

The primary goal of this study is to elucidate the role of microorganisms involved in redox transformations of arsenic and selenium in soils and sediments. In the absence of oxygen, microorganisms use a wide range of terminal electron acceptors from nitrate through iron, sulphate and carbonate for their respiration. Recent evidence indicates that there are microorganisms that exist in nature which are capable of utilizing arsenate or selenate for respiration by the process of dissimilatory arsenate or selenate reduction (Stolz and Oremland, 1999).

Specific objectives of this study

Of particular interest are the microbial transformations that occur in the anaerobic zone because these are central in determining the mobility of arsenic and selenium in the environment.

The main objectives and some questions that are be addressed in this study are:

- How diverse are the microorganisms that have the capability to carry out dissimilatory arsenate or selenate reduction and how widely are they present in the environment, in particular New Jersey where arsenic rich soils are found.
- Is the reduction of arsenate and selenate coupled to respiration in these organisms?
- How do other electron acceptors such as nitrate compete for carbon source in the same environment?
- What is the metabolic diversity of arsenate and selenate reducing bacteria in terms of carbon requirements?

Methodology

1. **Sampling:** Sediment grab samples were taken in the Meadowlands regions along the Hackensack River, NJ, from Sawmill Creek and Kearny Marsh. Sediments associated with vegetation from the two primary wetland plants *Spartina* sp. and *Phragmites* sp. and also an unvegetated mudflat were taken as a transect along the river, to determine the impact that vegetation may have on anaerobic reduction of arsenate and selenate. Plastic jars were filled to capacity with sediment, sealed and brought to the lab and stored at 4°C until used.
2. **Enrichment setup:** To determine the potential for dissimilatory selenate or arsenate reduction in the sediment, microcosms were setup with 10% w/v of sediment as an inoculum following strict anaerobic techniques in mineral salts media with 10 mM sodium selenate or 10 mM sodium arsenate as a terminal electron acceptor and 5 mM pyruvate and 200 μM 4-hydroxybenzoate as electron

donor. The microcosms were incubated at 27°C and sampled periodically. Samples were filtered through 0.4μm syringe filter and stored at –20°C until analyzed.

3. **Growth experiments with bacterial strain AK4OH1**: Strain AK4OH1 capable of selenate reduction was isolated from Arthur Kill (Knight et al. 2002). Cells grown in batch cultures were washed, centrifuged and re suspended in buffer and was used for growth experiments. Stoichiometry of selenate reduction was determined by incubating the cultures with and without electron donor or acceptor, to prove growth coupled to selenate respiration.
4. **Analytical techniques:** The selenium oxyions were analyzed using ion chromatography with an AS14 (Dionex) column and conductivity detector. The mobile phase was 3.5mM sodium carbonate and 1.5mM sodium bicarbonate at a flow rate of 1.5mL/min. The benzoates were analyzed with a high performance liquid chromatography system with C18 column (Phenomenex) and UV detection. The mobile phase was methanol:water:acetic acid in the ratio 60:58:2 at 1mL/min flow rate. Biomass was measured as increase in protein concentration according to Bradford's assay using Bio-Rad protein assay kit.
5. **DNA analysis:** The 16S rRNA gene from AK4OH1 was amplified and sequenced using eubacterial primers. Phylogenetic trees were constructed using Vector NTI suite 6.1.

Principal Findings and Significance

I. Physiological characterization of bacterial strain AK4OH1 capable of dissimilatory selenate reduction

Strain AK4OH1 is an anaerobic selenate reducing bacterium previously isolated from Arthur Kill, an intertidal strait between NY-NJ harbors (Knight et al. 2002). The present

work aims to characterize this strain and elucidate its capability to respire 4-hydroxybenzoate coupled to selenate reduction to selenite.

A. Growth coupled to selenate reduction

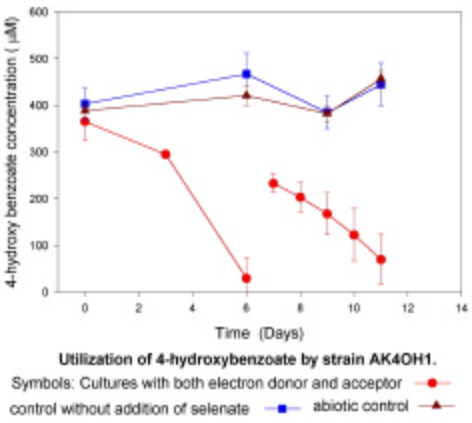

Figure1

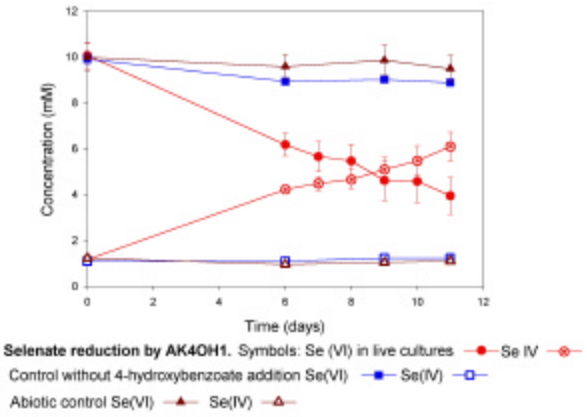

Figure2

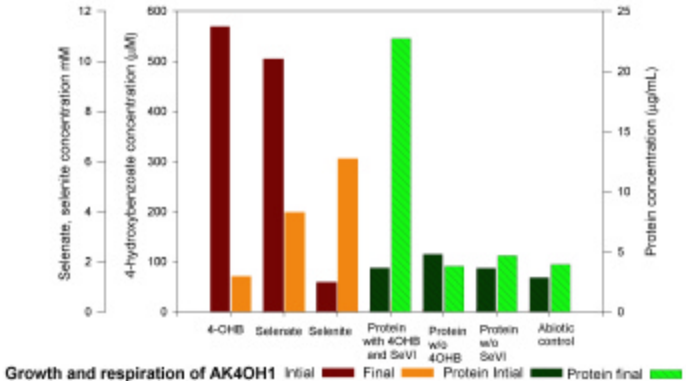

Figure 3

Figures 1, 2 and 3 shows that selenate is reduced to selenite with 4-hydroxybenzoate utilization. Protein concentration increased only when both electron acceptor and donor were present. Table 1 shows the final electron balance when AK4OH1 was incubated in the presence of both electron donor and acceptor.

Table 1 Balance of electron donors and acceptors during the utilization of 4-hydroxybenzoate by strain AK4OH1

System	Substrate utilized (μM)	Protein increase (μg/mL)	Carbon conversion to cell (%)[1]	Se (VI) reduced (mM)	Se (IV) produced (mM)	Electrons produced[2] (μmoles)	Electrons consumed[3] (μmoles)	Electron balance (%)
4-OHB and Se (VI)	524.53	19	43	5.73	4.75	209	236	113
W/O Se (VI)	0.45	0	0	NA	NA	12.6	NA	NA
W/O 4OHB	NA	0	0	0.0356	0.0056	NA	0.1312	NA

[1]The increase in cell carbon was estimated to be equal to increase in protein concentration.

[2]Calculated on the basis of stoichiometric equation:

$$C_7H_6O_3 + 11H_2O \longrightarrow 7CO_2 + 28H^+ + 28e^-$$

[3]Calculated on the basis of reduction of electron acceptor as follows:

$$SeO_4^{2-} + H_2O + 2e^- \longrightarrow SeO_3^{2-} + 2OH^-$$

$$SeO_3^{2-} + 3H_2O + 4e^- \longrightarrow Se^0 + 6OH^-$$

B. Metabolic diversity of AK4OH1

Table 2

Compounds	Growth with protein increase
Electron donors	
4-hydroxybenzoate	+
3-hydroxybenzoate	+
Benzoate	+
Acetate	+
Lactate	+
Electron acceptors	
Nitrate	+
Selenate	+
Sulfate	-
Arsenate	-

Table 2 summarizes the various electron donors and acceptors that AK4OH1 can utilize. Growth was considered positive only when there was loss of the compound accompanied with increase in protein concentration

C: Phylogenetic Analysis

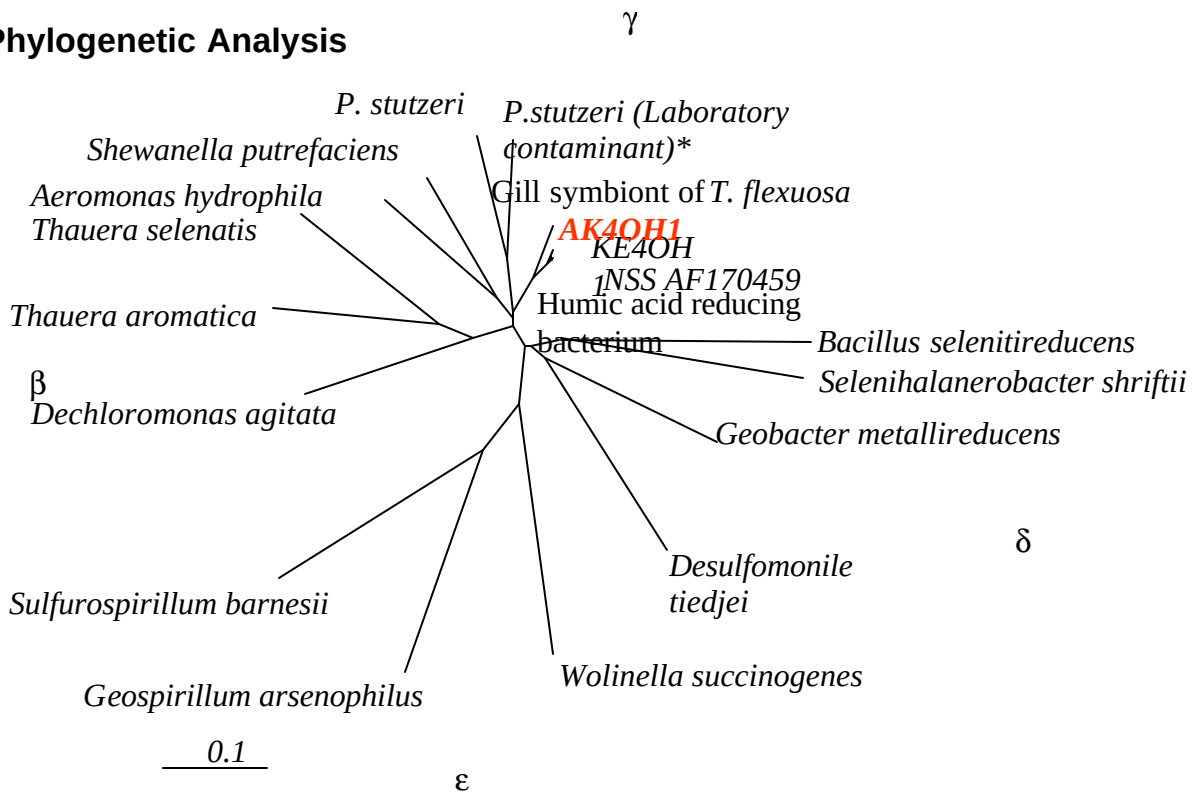

Figure 4

Based on 16S rRNA gene sequence, a phylogenetic tree was constructed shown in figure 4. It shows the relationship of AK4OH1 with other selenate reducers and their closely related species.

Conclusions

Bacterial strain AK4OH1 can be classified as a new genus and species based on phylogenetic sequence analysis of 16S rRNA gene. It clusters with a group of uncultured sulfur-oxidizing symbionts of bivalves and its closest relatives are, a humic acid reducing

bacterium and a perchlorate reducing strain with 99% and 97% similarity respectively. Strain AK4OH1 is capable of selenate reduction to selenite coupled to respiration and growth utilizing 4-hydroxybenzoate as sole carbon and energy source. In addition to selenate, nitrate can also serve as an electron acceptor for the growth of strain AK4OH1 but not sulfate or arsenate. The strain can also utilize other aromatic compounds such as 3-hydroxybenzoate and benzoate and short chain fatty acids such as acetate.

II. Sediment microcosms:

Figure 5: Selenate reducing enrichments showing a red precipitate indicating the formation of elemental selenium, autoclaved controls shows no selenate transformation

Table 3: Microcosms setup with sediments from various sampling sites showing selenate reducing potential

Sampling site	Selenate reducing potential
Sawmill creek- Phragmites	+
Sawmill creek -Spartina	+
Sawmill creek- Mudflat	+
Kearny Marsh	+
Chesequake- Phragmites	+
Chesequake- Spartina	+

Initial enrichment with the sediments from various regions in NJ showed that selenate could be readily transformed to selenite and even to elemental selenium. Subsequent transfers have been made and monitored for activity of the sediments. Future studies will focus on demonstrating activity in sequential transfers and isolation of pure cultures. The analysis of microcosms fed with arsenate is currently in progress.

References

1. Stolz, J.F and Oremland. R.S. (1999). Bacterial respiration of arsenic and selenium. FEMS Microbiol Reviews. 23: 615-627.
2. Knight V.K, Nijenhuis, I., Kerkhof, L.J., Häggblom, M.M. (2002). Degradation of aromatic compound coupled to selenate reduction. Geomicrobiology Journal. 19:77-86